

MAR 29 2007

Application No.: 09/980,542

Docket No.: ICON 3.3-001

IN THE SPECIFICATION

Please amend the paragraphs beginning on page 1, line 10 and ending on page 1, line 29:

Methodologies have evolved during the last twenty years to genetically engineer plants. In general, they are based on either direct DNA introduction into plant cells or indirect transfer mediated by ~~Agrobacterium~~ Agrobacterium tumefaciens. Methods involving direct transfer include particle bombardment of cultured plant tissues and DNA introduction into naked plant cells i.e., protoplasts, using polyethylene glycol or electroporation. See, e.g., Sawabel & Cove, *Biotech. Adv.* 10:394-412 (1992); Christou, *Cur. Opinion Biotech.* 4:135-141 (1993); Gelvin, *Cur. Opinion Biotech.* 9:227-232 (1998) and Birch, *Annu. Rev. Plant Physiol. Plant Mol. Biol.* 48:297-326 (1997). Most methods are variety-specific because they are based on use of *in vitro* grown regenerable plant systems which in turn are variety-specific. Except for a few economically important crops such as potato, tomato and canola, transformation methods available currently work with only a handful of varieties.

The traditional backcross method of breeding has provided a mechanism for the transfer of a trait from one line (the donor) to another line (the recurrent parent). See, e.g., Harlan and Pope, *J. Heredity*, 13:319-322 (1922). It has been particularly useful for corn, soybean and cotton. Successful backcross breeding requires: a previously derived recurrent parent; maintenance of the trait of interest during selection; and sufficient backcrosses to reconstitute the genome of the recurrent parent. Allard, *Principles of Plant Breeding*, Wiley and Sons (1960). During the backcross program, the hybrid population becomes increasingly homozygous for genes of the

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recurrent parent at a rate described by the formula:

$$\text{Proportion of homozygosity} = 1 - 0.5^n$$

Please amend the paragraph beginning on page 4, line 32 and ending on page 5, line 3:

selecting progeny of the regenerated plants that contain the heterologous nucleic acid. The fused cells or protoplasts *per se*, are also provided. Further, the methods of the present invention produce plants that have a different genetic make-up than transgenic plants made by other methods because the end result of the process is an individual plant that is genetically devoid of any resident DNA of primary transformant (i.e., the donor). Progeny of the plant, plant parts and seed and seed parts from the plant are also provided.

Please amend the paragraph beginning on page 6, line 5 and ending on page 6, line 14:

Species-specific chromosome elimination (genome segregation) in interspecific/intergeneric hybrids is a well-documented phenomenon. In many cases, however, unstable hybrids were of limited interest as the main breeding efforts were aimed at chromosome exchange between two parental genomes as a method for introgression of alien chromosomal material. Prior to the time the present invention was made, unstable hybrids segregating parental genomes were described only in terms of systems that produce haploid plants (interspecific, intergeneric crosses for production of haploid wheat, barley, or potato) or in terms of negative results of attempts to achieve an introgression of chromosome material from wild species into cultivated crops (such as from *Tripsacum* to maize or *Glycine tomentella* to soybean).

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Please amend the paragraph beginning on page 6, line 11 and ending on page 6, line 14:

Species-specific chromosome elimination (genome segregation) in interspecific/intergeneric hybrids is a well-documented phenomenon. In many cases, however, unstable hybrids were of limited interest as the main breeding efforts were aimed at chromosome exchange between two parental genomes as a method for introgression of alien chromosomal material. Prior to the time the present invention was made, unstable hybrids segregating parental genomes were described only in terms of systems that produce haploid plants (interspecific, intergeneric crosses for production of haploid wheat, barley, or potato) or in terms of negative results of attempts to achieve an introgression of chromosome material from wild species into cultivated crops (such as from *Tripsacum* to maize or *Glycine tomentella* to soybean).

Please amend the paragraph beginning on page 16, line 13 and ending on page 16, line 16:

a) *dSpm* contains NOS promoter separated by RS site (recombination site recognized by R recombinase from *Z. rouxii*) from the terminator of transcription of OCS gene. (See: pIC156 and pIC216, Figs. 1 and 2).

b) *dSpm* contains *pNOS:BAR-OCS3'*, (pIC312, pIC31A2, Figs. 3 and 4).

c) *dSpm* contains *pNOS:BAR-OCS3'*, but *BAR* gene is flanked by two unidirectional RS sites (pIC401, pIC411, Figs. 5 and 6).

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Please amend the paragraph beginning on page 16, line 20 and ending on page 17, line 5:

Seed of *Orychophragmus violaceus* were sterilized and germinated in vitro. The transformation of in vitro grown plants of the species has been done as previously described for *Brassica* species (De Block, et al., *Plant Physiol.*, 91, 694-701 (1989). The constructs used were *Agrobacterium*-based carrying *Spm* transposase along with different versions of non-autonomous *dSpm* element inserted between 35S CaMV promoter and GUS gene (see Fig.1). The plasmids were used to produce transformed *Orychophragmus* plants. Several transgenic plants have been produced and characterized. Two independent transformants containing a single copy insertion have been crossed as male parents to different *Brassica* species (*B.nigra*, *B.juncea*, *B.napus*, *B.carinata*) and *Sinapsis alba* as previously described. In total, approximately 600 crosses were done. The resultant hybrids were allowed to self and the F_1 progeny ~~has been~~ were selected for the presence of *dSpm* element (PCR analysis or phosphinotricin resistance). Those surviving selection were further screened for pure *Brassica* phenotype and for absence of GUS activity, and, finally, tested for absence of either transposase sequences, or species-specific *Orychophragmus* repeats. Finally, co-segregation of *dSpm* with a *Brassica* chromosome-specific RFLP pattern ~~has been~~ was established by analyzing the F_2 progeny.